



Effect of chrysanthemum flower extract on analgesia and serotonin levels associated with migraine symptoms in rats

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Objectives: The flower from chrysanthemum (*Chrysanthemum indicum* L.; CI) has been used in China as traditional Chinese medicine (TCM) for various symptoms; for example, hypertension, vertigo and infections. It has high potencies in several pharmacological effects including anti-inflammatory, antipyretic and analgesic activities. Serotonin (5-HT) is found in both central and peripheral nervous system. It plays a role in the process of pain, especially, severe pain such as migraine. Therefore, the purpose of this study aimed to evaluate the effect of CI on pain relieving and brain 5-HT in rat model.

Methods: The ethanolic extract of white chrysanthemum flower was used in this study. Rats were pretreated with CI at 250, 500, and 750 mg/kg, 200 mg/kg acetaminophen, and 100 mg/kg ibuprofen compared with vehicle (control). Tail-flick meter was used to determine analgesic effect. Comparisons of tail-flick latency and % maximal possible effect (MPE) among groups were evaluated. The dose of CI that produced the highest activities in both tail-flick latency and %MPE was then selected to study in reserpine-induced low 5-HT experiment compared with sumatriptan, a standard drug for the migraine treatment, to examine the brain 5-HT.

Results: The results showed that 500 mg/kg CI significantly possessed analgesic activity in rats as well as acetaminophen and ibuprofen, standard painkillers, did. Therefore, CI at this dose was chosen to study in reserpine-rat experiment. The study demonstrated that CI500 statistically boosted the brain 5-HT levels in reserpine-induced low 5-HT rats. An increase in the brain 5-HT was also found in rats treated with sumatriptan, a 5-HT agonist. It was possible that CI acted as "5-HT agonist" similarly to sumatriptan.

Conclusion: These findings suggest that the effect of CI on the minimization of algesia is possibly involved in serotonergic system. As it affected both pain behavior and brain 5-HT level, CI could be useful to be developed as an alternative antihyperalgesic substance, particularly migraine.

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Introduction

Chrysanthemum indicum L. (chrysanthemum; CI) has been used in China as a traditional Chinese medicine (TCM) for various symptoms; for example, hypertension, vertigo, and infections¹. The flower from CI was found to contain chrysanthemin, adenine, choline, stachydrine, luteolin and volatile oils such as borneol, chrysanthenone and bornyl acetate². It has high potencies in several pharmacological effects including anti-inflammatory, antipyretic, and analgesic activities^{3,4}. Serotonin (5-HT) is found in both central and peripheral nervous system. It plays a role in the process of pain, especially, severe pain such as migraine⁵. Decrease in 5-HT levels is found during migraine attacks⁶. Hence, one of mechanisms for migraine treatment is increasing 5-HT level (5-HT agonist) in order to relieve this severe pain. Therefore, the purposes of this study were to evaluate the effect of CI on pain relieving and to determine the brain 5HT level in reserpine-induced low 5-HT rat.

Methods

Sixty male Wistar rats were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom. They were acclimatized under standard conditions for 1 week with free access of food and water. All procedures and animal care were approved by Institutional Animal Care and Use Committee of Thailand Institute of Scientific and Technological Research.

The ethanolic extract of CI flower was prepared in 12% w/v of acacia solution, which was used as a vehicle, at 125 mg/ml. For analgesic experiment, acetaminophen and ibuprofen, standard painkillers, were also prepared in the vehicle at 50 and 25 mg/ml, respectively. For reserpine-induced low 5-HT in rat model, reserpine (Sigma, USA) was dissolved in

0.9% NSS at 0.2 mg/ml. Sumatriptan (Imigran™; GlaxoSmithKline, Poland), a standard drug in 5-HT agonist group, was prepared in 12% w/v of acacia solution at 25 mg/ml. Serotonin Research ELISA™ kit (Labor Diagnostika Nord GmbH, Germany) was used to determine 5-HT levels in the brain. Tail-flick meter (Ugo Basile, Italy) was used to study analgesic effect in rats.

Analgesic effect of CI

The analgesic activity of CI in rats was determined using tail-flick meter. Thirty six rats were randomly divided into 6 groups (6 rats of each) to orally receive test substances as the following:

1. Control group: 12% w/v of acacia solution as a vehicle (Control)
2. Standard group 1: 200 mg/kg acetaminophen in vehicle (STD1)
3. Standard group 2: 100 mg/kg ibuprofen in vehicle (STD2)
4. Treatment group 1: 250 mg/kg CI in vehicle (CI250)
5. Treatment group 2: 500 mg/kg CI in vehicle (CI500)
6. Treatment group 3: 750 mg/kg CI in vehicle (CI750)

Antinociception effect was evaluated as response latencies of the rat that flicked its tail after exposing a light heat source underneath the platform of tail-flick meter, where the animal was placed. The maximum allowable latency, or cut-off time, was automatically set at 20 seconds (s) by the apparatus to prevent any tissue damage. The intensity of the light was set at intermediate level, 50, so that tail-flick baseline latencies of thrice pretreatment values were averaged between 2.0 and 2.5 s. Rats were assessed the tail-flick method⁷⁻⁹ immediately after administration (0 min), and were periodically measured with a 30-min interval for 2 hr and a 60-min interval for another 2 hr. Analgesic activity was expressed as a percent maximal possible effect (% MPE), which was calculated as follows:

$$\% \text{ MPE} = [(\text{test latency} - \text{baseline latency}) / (20 - \text{baseline latency})] \times 100; \text{ where 20 is cut-off time in seconds.}$$

The dose of CI that exhibited the best analgesic effect was selected to study in the reserpine-induced low 5-HT test.

CI effects on the brain 5-HT in reserpine rats

The method of reserpine-induced low 5-HT in the rat model¹⁰ was performed in twenty four rats. They were randomly divided into 4 groups with 6 rats of each to orally receive test substances as the following:

1. Sham group: (Sham)
2. Control group: 12% w/v of acacia solution as a vehicle (Control)
3. Standard group: 100 mg/kg sumatriptan in vehicle (SUM)
4. Treatment group: 500 mg/kg CI in vehicle (CI500)

Rats were pretreated with 0.25 mg/kg reserpine via intraperitoneal injection (i.p.) daily for 9 days, except the sham group which was received 0.9% NSS. From the 3rd to the 9th day of the injection, rats were orally received corresponding test substances as above 30 min before reserpine administration. On the last day of the experiment (day 9), 30 min after reserpine injection, rats were sacrificed to collect the brain for determining 5-HT levels. The brain was prepared. Briefly, the brain was homogenized in radio immune precipitation assay (RIPA) buffer containing protease inhibitor and were then added 1% stabilizer to prevent 5-HT denaturation¹¹. The brain 5-HT was determined according to the manufacturer's instructions of a serotonin research ELISA kit.

Statistical analysis

All data were expressed as mean \pm standard error. Statistical analysis for comparing treatment effects of each group of rats was done by one-way ANOVA. Comparisons among groups were conducted using the LSD post-hoc analysis. Student's t-test techniques were also used. Statistical significance was defined as $p < 0.05$.

Results

Analgesic effect of CI

Analgesic activity of each treatment is expressed as tail-flick latencies and % MPE at the indicated time points. After oral administration, latency of tail flicking was immediately performed in order to ensure to see the onset of analgesic effect. The comparisons of tail-flick latencies and % MPE among the treatment groups were shown in Table 1 and Figure 1, respectively. The results revealed that CI500 significantly exhibited the longest latencies with the highest % MPE starting at 60 min and long-lasting to 120 min after administration. Therefore, 500 mg/kg CI was selected to study its effects on the brain 5-HT in reserpine-induced low 5-HT test. For standard pain-killer drugs, both STD1 and STD2 significantly started analgesic activity at 60 min, but did not show longer periods of effective time than all CI groups did.

Table 1 Analgesic activity shown in latencies of flicking the rat's tail at different intervals after oral administration of 3 doses of CI, standard drug 1 (STD1), and 2 (STD2) compared with the control group in the tail-flick test; * = $p < 0.05$ when compared with the control group.

Group	Latency (s)						
	0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control	3.9 \pm 0.3	3.4 \pm 0.3	2.8 \pm 0.4	2.8 \pm 0.4	3.1 \pm 0.1	3.0 \pm 0.4	2.8 \pm 0.4
STD1	4.2 \pm 0.5	5.3 \pm 1.1	4.2 \pm 0.5*	4.1 \pm 0.5*	3.6 \pm 0.4	3.7 \pm 0.3	3.1 \pm 0.4
STD2	2.8 \pm 0.4	3.1 \pm 0.2	4.1 \pm 0.5*	3.6 \pm 0.3	3.5 \pm 0.4	3.9 \pm 0.4	3.9 \pm 0.4*
CI250	3.9 \pm 0.3	3.4 \pm 0.2	3.8 \pm 0.3	4.2 \pm 0.3*	3.7 \pm 0.3	4.1 \pm 0.4	3.5 \pm 0.1
CI500	3.9 \pm 0.2	3.9 \pm 0.4	4.5 \pm 0.5*	4.1 \pm 0.2*	4.9 \pm 0.4*	4.1 \pm 0.4	3.3 \pm 0.2
CI750	4.3 \pm 0.4	4.8 \pm 0.7	4.0 \pm 0.3*	4.1 \pm 0.5*	3.9 \pm 0.4	3.8 \pm 0.7	3.4 \pm 0.2

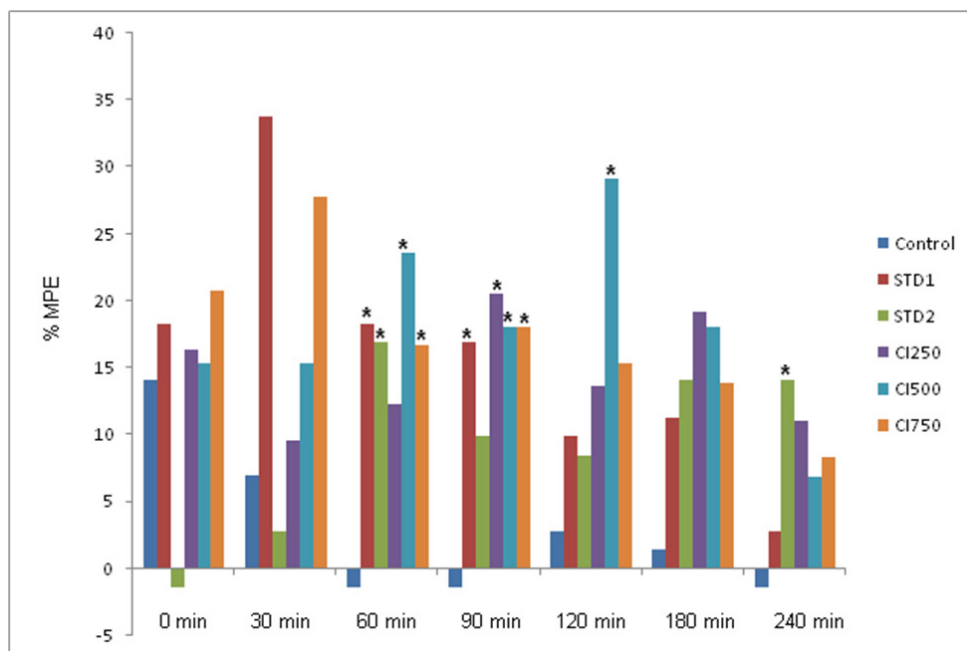


Figure 1 Analgesic activity at different intervals after administration of 3 doses of CI (CI250, CI500, and CI750), standard drug 1 (STD1), and 2 (STD2) compared with the control group (control) in the tail-flick test in rats. The results are expressed as percent of maximal possible effect (% MPE) with statistical significance at $p < 0.05$ (*).

CI effects on the brain 5-HT in reserpine rats

Reserpine produced statistically low 5-HT in the brain of the control rats compared with the sham group as shown in Figure 2. Meanwhile, 5-HT levels were significantly increased in CI500 group as well as in SUM rats.

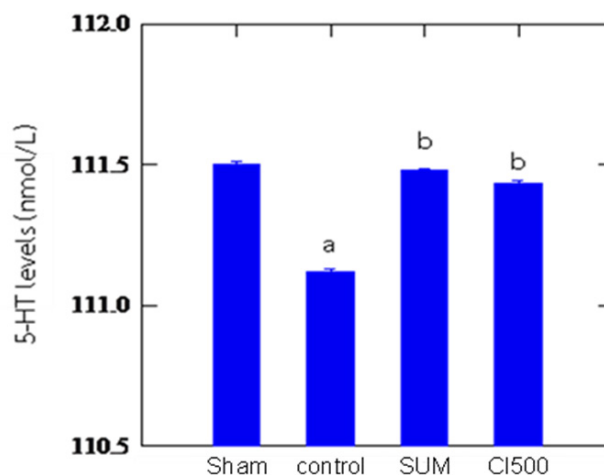


Figure 2 5-HT levels after administration of 500mg/kg CI (CI500) and sumatriptan (SUM) compared with the control group (control) and sham group (sham) in reserpine-induced low 5-HT rat test. Whereas a = $p < 0.05$ when compared with the sham group, and b = $p < 0.05$ when compared with the control group.

Discussion

500 mg/kg CI significantly possessed analgesic activity in rats as well as acetaminophen and ibuprofen, standard painkillers, did. Moreover, CI at this dose statistically boosted the brain 5-HT levels in reserpine-induced low 5-HT rats. An increasing in the brain 5-HT was also found in rats treated with sumatriptan, a 5-HT agonist. It was possible that CI acted as “5-HT agonist” similarly to sumatriptan.

Conclusion

These findings suggest that the effect of CI on the minimization of algnesia is possibly involved in serotonergic system. As it affected both pain behavior and brain 5-HT level, CI could be useful to be developed as an alternative antihyperalgesic substance, particularly migraine.

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